

Figure 1/7

Nucleotide Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5):

SEQ ID No.1

atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt 48  
gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag 96  
ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc 144  
act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc 192  
tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cg 240  
cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga 288  
act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc 336  
aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att 384  
gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432  
tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480  
atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528  
caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576  
gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624  
aaa gat ccc aac gaa aag aga gac cac atg gtc ctt gag ttt gta 672  
aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa tag 717

Figure 2/7

Amino Acid Sequence of wtGFP (Chalfie et al, Science, (1994), 263, 802-5

SEQ ID No.2

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val  
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu  
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys  
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe  
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg  
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn  
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly  
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val  
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val  
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

Figure 3/7

Predicted Amino Acid Sequence of F64L-S175G-E222G-GFP:  
SEQ ID No.3

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val  
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu  
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys  
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu  
50 55 60

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg  
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn  
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly  
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val  
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Gly Phe Val  
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

Figure 4/7

Predicted Amino Acid Sequence of F64L-S65T-S175G-GFP:  
SEQ ID No.4

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val  
1 5 10 15

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu  
20 25 30

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys  
35 40 45

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu  
50 55 60

Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg  
65 70 75 80

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
85 90 95

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
100 105 110

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn  
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly  
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val  
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val  
210 215 220

Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

Figure 5/7

Flow cytometry of GFP mutations

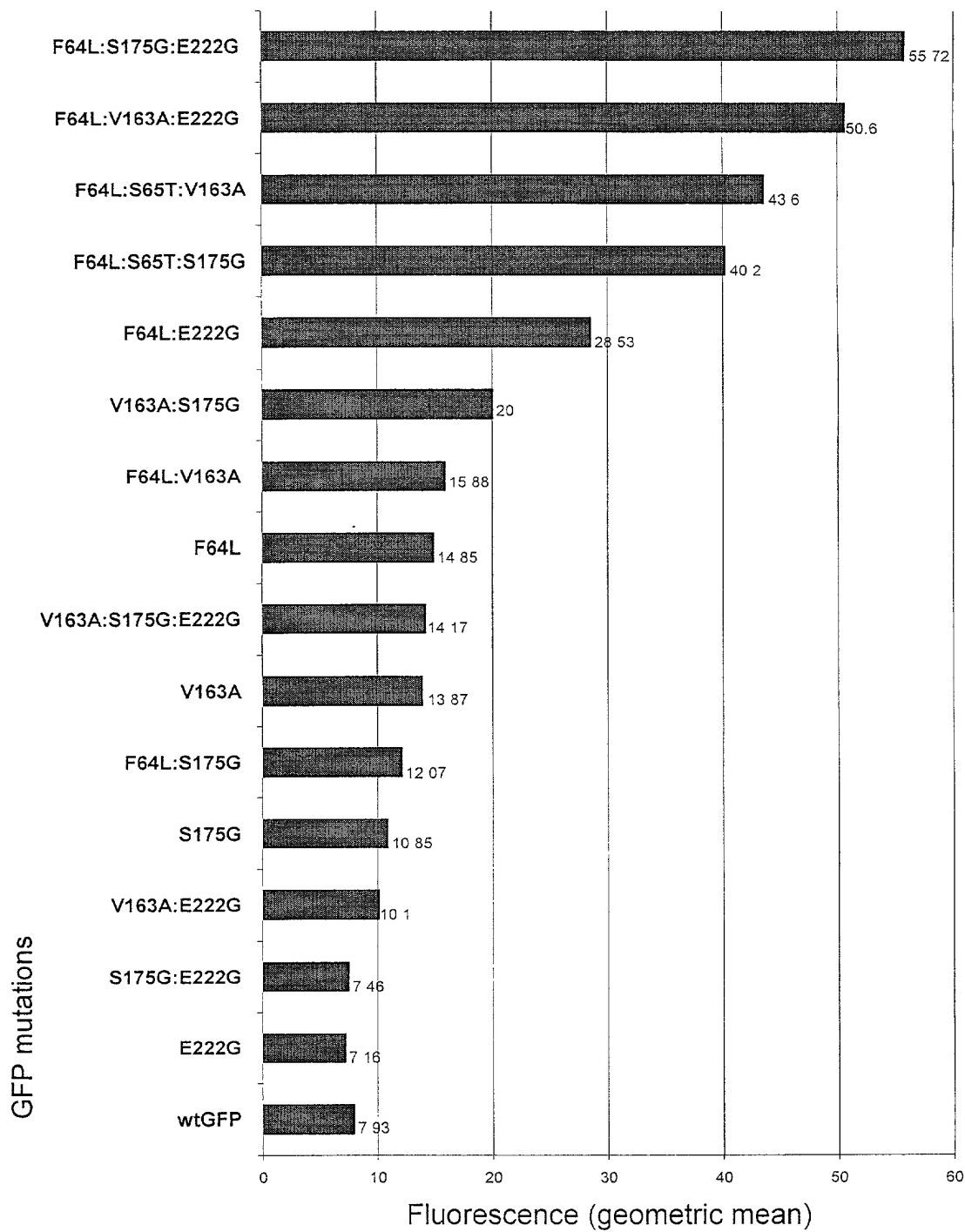


Figure 6/7

Photobleaching of GFP mutations

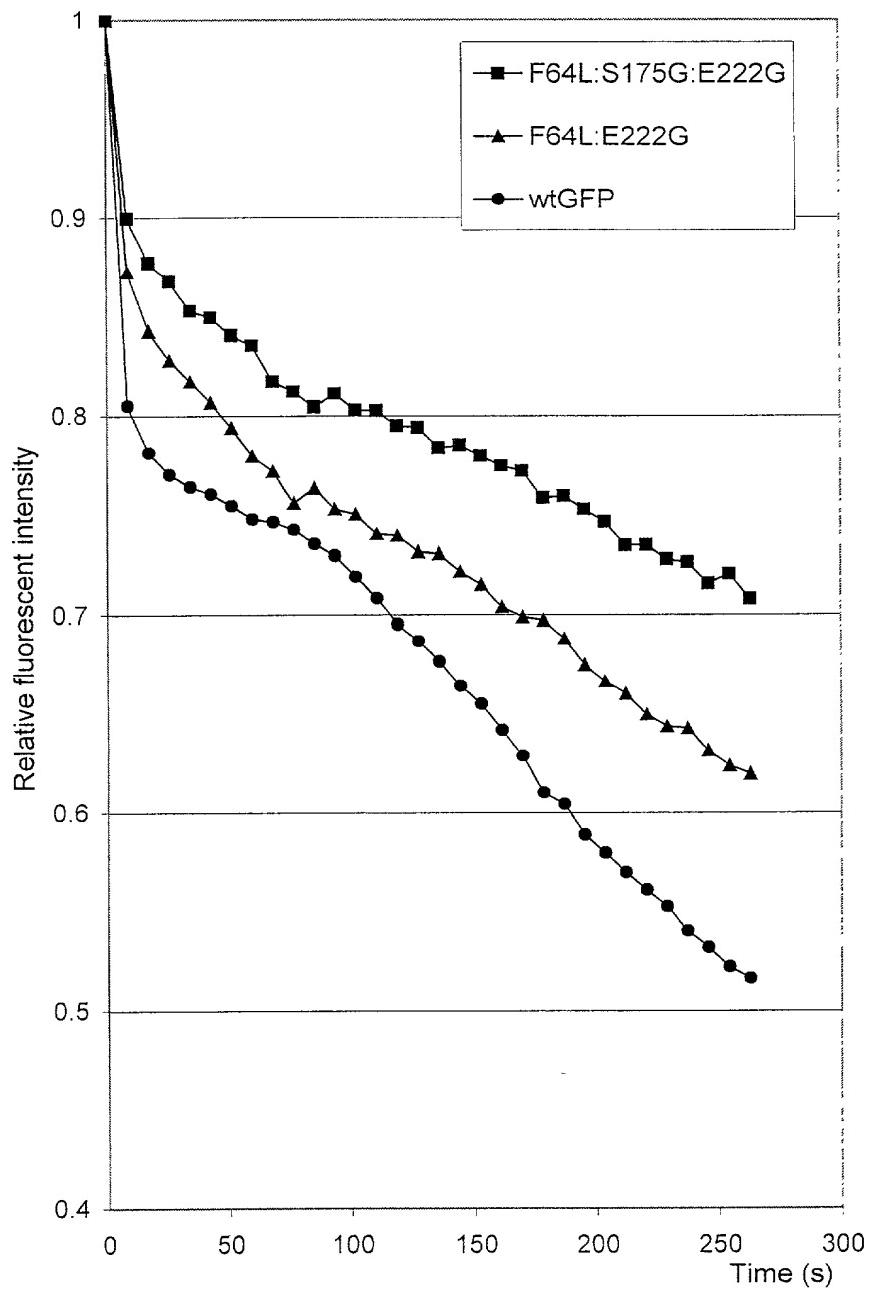


Figure 7/7

Live Cell CHO-HIR NF<sub>κ</sub>B Assay  
P65-tri GFP Assay T = 30 mins

